

## RAFFINOSE SYNTHASE GENES AND THEIR USE

### BACKGROUND OF THE INVENTION

#### 5 FIELD OF INVENTION

The present invention relates to raffinose synthase genes and their use.

#### DISCLOSURE OF THE RELATED ART

10 Raffinose family oligosaccharides are derivatives of sucrose, which are represented by the general formula: o- $\alpha$ -D-galactopyranosyl-(1 $\rightarrow$ 6)<sub>n</sub>-o- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-fluctofuranoside, and they are called "raffinose" when n is 1, "stachyose" when n is 2, "verbascose" when n is 3, and "ajugose" when n is 4.

15 It has been known that raffinose family oligosaccharides have an effect of giving good conditions of enterobacterial flora, if present at an appropriate amount in food. Therefore, raffinose family oligosaccharides have already been used as a functional food material for addition  
20 to some kinds of food and utilized in the field of specific health food. On the other hand, raffinose family oligosaccharides are neither digested nor absorbed in mammals such as human, but are assimilated and decomposed by enterobacteria to generate gases and to cause meteorism and absorption disorder. Therefore, it  
25 has been desired to appropriately regulate the amount of raffinose family oligosaccharides in food and feed.

Raffinose family oligosaccharides are synthesized by the raffinose family oligosaccharide biosynthesis system beginning with sucrose in many plants. This biosynthesis system normally involves a reaction for the sequential addition of galactosyl groups from galactinol through an  $\alpha$  (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule. Raffinose synthase is the enzyme concerned in the reaction for producing raffinose by allowing a D-galactosyl group derived from galactinol to form the  $\alpha$  (1 $\rightarrow$ 6) bond with the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule in the first step of this biosynthesis system. It has been suggested that this enzyme constitutes a rate-limiting step in the above synthesis system, and therefore this enzyme is quite important in the control of biosynthesis of raffinose family oligosaccharides.

Then, a method for controlling an expression level or activity of raffinose synthase in plants by utilizing a raffinose synthase gene is effective to control a biosynthesis system of raffinose family oligosaccharides in plants to increase or decrease the production of raffinose in plants.

Thus, a raffinose synthase gene which can be used in such a method has been desired.

The main object of the present invention is to provide novel raffinose synthase genes from plants.

This object as well as other objects and advantage of the present invention will become apparent to those skilled  
5 in the art from the following description.

#### SUMMARY OF THE INVENTION

Under these circumstances, the present inventors have intensively studied and succeeded in isolating novel genes  
10 encoding raffinose synthase from various plants. Thus, the present invention has been completed.

That is, the present invention provides:

1. A raffinose synthase gene which comprises a nucleotide sequence hybridizable with a nucleotide sequence  
15 selected from the group consisting of:

(a) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1,

(b) the nucleotide sequence represented by SEQ ID NO:  
2,

20 (c) a nucleotide sequence encoding the amino acid sequence of represented by SEQ ID NO: 3,

(d) the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4,

25 (e) a nucleotide sequence encoding the amino acid

sequence represented by SEQ ID NO: 5,

(f) the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6,

5 (g) a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7, and

(h) the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8,

10 under stringent conditions, and encoding a protein being capable of binding D-galactosyl group through  $\alpha$  (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in a sucrose molecule to form raffinose.

2. A raffinose synthase gene comprising a  
15 nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1.

3. A raffinose synthase gene comprising the nucleotide sequence represented by SEQ ID NO: 2.

4. A raffinose synthase gene comprising a  
20 nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 3.

5. A raffinose synthase gene comprising the nucleotide sequence represented by the 236th to 2584th nucleotides in the nucleotide sequence represented by SEQ ID  
25 NO: 4.

6. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 5.

5 7. A raffinose synthase gene comprising the nucleotide sequence represented by the 134th to 2467th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6.

10 8. A raffinose synthase gene comprising a nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 7.

9. A raffinose synthase gene comprising the nucleotide sequence represented by the 1st to 1719th nucleotides in the nucleotide sequence represented by SEQ ID NO: 8.

15 10. A raffinose synthase gene comprising the nucleotide sequence represented by SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

11. A nucleic acid comprising a partial nucleotide sequence of the raffinose synthase gene of any one of the above 1 to 10.

20 12. A method for detecting a nucleic acid containing a raffinose synthase gene which comprises detecting said nucleic acid by hybridization using the labeled nucleic acid of the above 11 as a probe.

25 13. A method for amplifying a nucleic acid containing a raffinose synthase gene which comprises amplifying

said nucleic acid by polymerase chain reaction (PCR) using the nucleic acid of the above 11 as a primer.

14. A method for obtaining a raffinose synthase gene which comprises the steps of:

5 detecting a nucleic acid containing said raffinose synthase gene by hybridization using the labeled nucleic acid of the above 11 as a probe, and

recovering the detected nucleic acid.

10 15. A method for obtaining a raffinose synthase gene which comprises the steps of:

amplifying a nucleic acid containing said raffinose synthase gene by PCR using the nucleic acid of the above 11 as a primer, and

recovering the amplified nucleic acid.

15 16. A nucleic acid comprising a nucleic acid containing the raffinose synthase gene of any one of the above 1 to 10 which is joined to a nucleic acid exhibiting promoter activity in a host cell.

20 17. A vector comprising the raffinose synthase gene of any one of the above 1 to 10.

18. A transformant, wherein the raffinose synthase gene of any one of the above 1 to 10 is introduced into a host cell.

25 19. A transformant, wherein the nucleic acid of the above 16 is introduced into a host cell.

20. A transformant, wherein the vector of the above 17 is introduced into a host cell.

21. The transformant of any one of the above 18 to 20, wherein the host is a microorganism.

5 22. The transformant of any one of the above 18 to 20, wherein the host is a plant.

23. A method for producing a raffinose synthase which comprises the steps of:

10 culturing or growing the transformant of any one of the above 18 to 22 to produce the raffinose synthase, and collecting the raffinose synthase.

24. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 1.

15 25. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 3.

26. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 5.

27. A raffinose synthase comprising the amino acid sequence represented by SEQ ID NO: 7.

20 The term "nucleic acid" used herein means an oligomer compound or a high molecular compound which is generally called "DNA" or "RNA".

#### DETAILED DESCRIPTION OF THE INVENTION

25 The gene engineering techniques described below can

be carried out, for example, according to methods described in  
"Molecular Cloning: A Laboratory Manual 2nd edition" (1989),  
Cold Spring Harbor Laboratory Press, ISBN 0-87969-309-6;  
"Current Protocols In Molecular Biology" (1987), John Wiley &  
5 Sons, Inc. ISBN 0-471-50338-X; "Current Protocols In Protein  
Science" (1995), John Wiley & Sons, Inc. ISBN 0-471-11184-8.

The genes of the present invention can be obtained  
from soybean, plants belonging to the families Chenopdiaceae  
such as beet, etc. and Cruciferae such as mustard, rapeseed,  
10 etc. Specific examples of the genes of the present invention  
include those comprising a nucleotide sequence encoding the  
amino acid sequence represented by SEQ ID NO: 1, the nucleotide  
sequence represented by SEQ ID NO: 2, a nucleotide sequence  
encoding the amino acid sequence represented by SEQ ID NO: 3,  
15 the nucleotide sequence represented by SEQ ID NO: 4 or by the  
236th to 2584th nucleotides in the nucleotide sequence  
represented by SEQ ID NO: 4, a nucleotide sequence encoding the  
amino acid sequence represented by SEQ ID NO: 5, the nucleotide  
sequence represented by SEQ ID NO: 6 or by the 134th to 2467th  
20 nucleotides in the nucleotide sequence represented by SEQ ID  
NO: 6, a nucleotide sequence encoding the amino acid sequence  
represented by SEQ ID NO: 7, the nucleotide sequence represented  
by SEQ ID NO: 8 or by the 1st to 1719th nucleotides in the  
nucleotide sequence represented by SEQ ID NO: 8, and the like.

25 The genes of the present invention can be obtained,



for example, by the following method.

That is, the genes of the present invention derived from soybean can be obtained, for example, by the following method.

5 For example, the gene can be obtained by a hybridization method using a nucleic acid having the nucleotide sequence represented by SEQ ID NO: 2 or its partial nucleotide sequence as a probe to detect a nucleic acid fragment which hybridizes to the probe in DNAs derived from soybean, followed  
10 by isolating the detected nucleic acid.

In this method, first, a nucleic acid to be used as the probe is prepared. As such a nucleic acid, for example, there is a nucleic acid composed of an oligonucleotide chemically synthesized by a conventional method on the basis of the  
15 nucleotide sequence of SEQ ID NO: 2. Specific example thereof includes a nucleic acid having the 800th to the 899th nucleotides in the nucleotide sequence represented by SEQ ID NO: 2.

Alternatively, the gene of the present invention derived from soybean can be obtained by the following method.

20 For example, tissue of soybean (*Glycine max*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in  
25 the extraction. RNA is recovered from thus-obtained RNA extract

by ethanol precipitation. Poly-A tailed RNA is fractionated from thus-recovered RNA by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation. cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 2. More specifically, as the primers, for example, there are primers 11 and 12 shown in List 1 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from soybean, the genes of the present invention derived from soybean, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 1" and the "raffinose synthase gene having the nucleotide sequence of SEQ ID No: 2" can be obtained.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

Alternatively, cloning can be carried out, for example, by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such

as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 1

- Primer 11: (SEQ ID NO: 9) ccaatctgat catgcttggtg ccgaa 25mer  
10 Primer 12: (SEQ ID NO: 10) ggaacaaagt tatgcactat tatttaaggt 30mer

The genes of the present invention derived from a Chenopdiaceae plant such as beet can be obtained by the following method.

For example, tissue of a Chenopdiaceae plant such as beet (*Beta vulgaris*) is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From this tissue debris powder, RNA is extracted by a conventional method. A commercially available RNA extraction kit can be utilized in the extraction. RNA is recovered from the thus-obtained RNA extract by ethanol precipitation. From the recovered RNA, poly-A tailed RNA is fractionated by a conventional method. A commercially available oligo-dT column can be utilized in this fractionation.

cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by

utilizing a commercially available cDNA synthesis kit. DNA is amplified by PCR using the above-obtained cDNA as the template and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. More specifically, as the primers, for example, there are primers 21 and 22 shown in List 2 hereinafter. When PCR is carried out by using these primers and as the template cDNA derived from beet, the genes of the present invention derived from beet, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 3," and the "raffinose synthase gene having a nucleotide sequence of SEQ ID No: 4" can be obtained.

According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 4. For example, in order to amplify the "raffinose synthase gene having the nucleotide sequence represented by the 236th to the 2584th nucleotides in the nucleotide sequence represented by SEQ ID NO: 4", preferably, oligonucleotides having the nucleotide sequences represented by primers 23 and 24 in List 2 below are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X.

Alternatively, cloning can be carried out by using a commercially available cloning kit such as TA cloning kit (Invitrogen) and a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined, for example, by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, a commercially available kit such as ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit manufactured by Perkin-Elmer can be used.

List 2

(SEQ ID NO: 11)  
Primer 21: ctaccaaatt ccacaactta aagttca 27mer  
(SEQ ID NO: 12)  
Primer 22: ggaataataa gcttcacaca tactgtactc tc 32mer  
(SEQ ID NO: 13)  
15 Primer 23: atggctccaa gctttagcaa ggaaaattcc 30mer  
(SEQ ID NO: 14)  
Primer 24: tcaaaataag tactcaacag tggtaaaacc 30mer

The genes of the present invention derived from Cruciferae plants such as mustard (*Brassica juncea*) and rapeseed (*Brassica napus*) can be obtained by the following method.

20 For example, tissue of a Cruciferae plant such as mustard or rapeseed is frozen in liquid nitrogen and ground physically with a mortar or other means into finely divided tissue debris powder. From the tissue debris powder, RNA is extracted by a conventional method. A commercially available

RNA extraction kit can be utilized in the extraction. The RNA is recovered from thus-obtained RNA extract by ethanol precipitation. Poly-A tailed RNA is fractionated from the RNA thus recovered by a conventional method. A commercially available oligo-dT column can be utilized in the fractionation.

cDNA is synthesized from the poly-A tailed RNA thus obtained by a conventional method. The synthesis can be carried out by using a commercially available cDNA synthesis kit. DNA are amplified by PCR using the above-obtained cDNA as a template and primers designed and chemically synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, when PCR is carried out by using cDNA derived from mustard (*Brassica juncea*) as the template and primers <sup>(SEQ ID NO: 7) (SEQ ID NO: 8)</sup> 33 and 34 shown in List 3 hereinafter, the genes from Cruciferae plants of the present invention, e.g., the "raffinose synthase gene having a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 5," and the "raffinose synthase gene having the nucleotide sequence represented by the 1st to 2654th nucleotides in the nucleotide sequence represented by SEQ ID NO: 6" can be obtained.

According to a particular purpose, the PCR primers can also be designed and synthesized on the basis of the nucleotide sequence of SEQ ID NO: 6. For example, in order to amplify DNA encoding the open reading frame region of the "raffinose synthase gene having a nucleotide sequence encoding a protein having the

amino acid sequence of SEQ ID NO: 5", and the "raffinose synthase gene having the nucleotide sequence represented by the 134th to the 2467th nucleotides of SEQ ID NO: 6", preferably, oligonucleotides having the nucleotide sequences represented by primers 35 and 36 in List 3 are synthesized and used as the primers.

The amplified DNA can be cloned according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; or "Current Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc. ISBN 0-471-50338-X. Alternatively, cloning can be carried out, for example, by using a commercially available TA cloning kit (Invitrogen) or a commercially available plasmid vector such as pBluescript II (Stratagene). The nucleotide sequence of the DNA clone can be determined by dideoxy terminating method such as that described by F. Sanger, S. Nicklen, A.R. Coulson, Proceedings of National Academy of Science U.S.A. (1977), 74, pp. 5463-5467. For example, preferably, the commercially available ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer can be used.

List 3

(SEQ ID NO: 15)  
Primer 31: ttggaagaga agacgccgcc gggaatcgtc 30mer  
(SEQ ID NO: 16)  
Primer 32: ttaagccccg gcgagagctc tggccggaca 30mer  
(SEQ ID NO: 17)  
Primer 33: accaatccaa aatctcatca aataatcgca 30mer

Primer 34: (SEQ ID NO:18) aaataatagg ggcagtacaa attacaccac 30mer  
Primer 35: (SEQ ID NO:19) atggctccac cgagcgtaat taaatccga 29mer  
Primer 36: (SEQ ID NO:20) ctaaaactca tacttaatag aagacaaacc 30mer

Then, a nucleic acid having a partial nucleotide  
5 sequence of the gene of the present invention (hereinafter  
referred to as "the gene fragment") which is obtained by the  
above-described method is labeled and then used as a probe in  
a hybridization method. The probe can be hybridized to, for  
example, DNA derived from soybean, a Chenopdiaceae plant or a  
10 Cruciferae plant to detect a nucleic acid having the probe  
specifically bound thereto, thereby detecting a nucleic acid  
having the raffinose synthase gene.

As the DNA derived from soybean, a Chenopdiaceae plant  
such as beet or a Cruciferae plant such as mustard or rapeseed,  
15 for example, a cDNA library or a genomic DNA library of these  
plants can be used. The gene library may also be a commercially  
available gene library as such or a library constructed according  
to a conventional library construction method, for example,  
described in "Molecular Cloning: A Laboratory Manual 2nd  
20 edition" (1989), Cold Spring Harbor Laboratory Press; "Current  
Protocols In Molecular Biology" (1987), John Wiley & Sons, Inc.  
ISBN 0-471-50338-X.

As the hybridization method, for example, plaque  
hybridization or colony hybridization can be employed, and they  
25 are selected depending upon the kind of vector used in the



construction of a library. More specifically, when the library to be used is constructed with a phage vector, a suitable host microorganism is mixed with the phage of the library under infectious conditions to obtain transformants. The

5 transformant is further mixed with a soft agar medium, and the mixture is plated on an agar medium. Thereafter, the mixture is cultured at 37°C until a plaque of an appropriate size appears.

When the library to be used is constructed with a plasmid vector, the plasmid is introduced into a suitable host microorganism to form transformants. The transformant obtained is diluted to a suitable concentration and the dilution is plated on an agar medium, after which it is cultured at 37°C until a colony of an appropriate size appears. In either case of the above

10 libraries, a membrane filter is placed on the surface of the agar medium after the above cultivation, so that the phage or transformant is transferred to the membrane. This membrane is denatured with an alkali, followed by neutralization, and for example, when a nylon membrane is used, the membrane is

15 irradiated with ultraviolet light, so that DNA of the phage or transformant is fixed on the membrane. This membrane is then subjected to a hybridization method wherein the gene fragment which has a partial nucleotide sequence of the gene of the present invention and labeled by a conventional method (hereinafter referred to as "the labeled gene fragment") is used as a probe.

25 For this method, reference may be made, for example, to D.M.

Glover ed., "DNA cloning, a practical approach" IRL PRESS (1985), ISBN 0-947946-18-7. There are various reagents and temperature conditions to be used in the hybridization. For example, in general, prehybridization is carried out by immersion of the  
5 membrane in a prehybridization solution [6 x SSC (0.9 M NaCl, 0.09 M citric acid), 0.1 to 1 (w/v)% SDS, 100 µg/ml denatured salmon sperm DNA] and incubation at 65°C for 1 hour. Then, hybridization is carried out by addition and mixing of the labeled gene fragment thereto and incubating the membrane at  
10 42 to 68°C for 4 to 16 hours.

In the present invention, the "stringent conditions" are those wherein incubation is carried out, for example, at 65 to 68°C in the above hybridization.

After hybridization, the membrane is taken out and  
15 is washed with 2 x SSC containing 0.1 to 1 (w/v)% SDS, further rinsed with 0.2 x SSC containing 0.1 to 1 (w/v)% SDS, and then dried. The membrane is analyzed, for example, by autoradiography or other techniques to detect the position of the probe on the membrane, thereby detecting the position on  
20 the membrane of a nucleic acid having a nucleotide sequence homologous to that of the probe used. The clone corresponding to the position of the nucleic acid thus detected on the membrane is identified on the original agar medium and the positive clone is selected so that the clone having the nucleic acid can be

isolated. The same procedures of detection are repeated to purify the clone having the nucleic acid.

Alternatively, a commercially available kit such as GENE TRAPPER cDNA Positive Selection System kit (GibcoBRL) can be used. In this method, first, a single-stranded DNA library is hybridized with the biotinylated gene fragment (i.e., probe), followed by adding streptoavidin-bound magnet beads and mixing.

From the mixture, the streptoavidin-bound magnetic beads are collected with a magnet, so that single-stranded DNA having a nucleotide sequence homologous to that of the probe used, which has been bound to these beads through the gene fragment, biotin and streptoavidin, is collected and detected. The single-stranded DNA collected can be converted into a double-strand form by reaction with a suitable DNA polymerase using a suitable oligonucleotide as a primer.

As described above, a nucleic acid containing raffinose synthase gene can be obtained by detecting a nucleic acid hybridizable to the gene fragment in DNAs of a gene library derived from soybean, a Chenopodiaceae plant or a Cruciferae plant, purifying a clone having the nucleic acid and isolating phage or plasmid DNA from the clone. By preparing the restriction map or determining the nucleotide sequence of the nucleic acid thus obtained according to a conventional method, the nucleic acid containing the gene of the present invention can be confirmed.

For example, the gene of the present invention from

a Chenopodiaceae plant can be confirmed by the following point:

The amino acid encoded by the nucleotide sequence thus determined has 75% or more homology to the amino acid sequence represented by the 103rd to 208th amino acids in the amino acid sequence of SEQ ID NO: 3;

80% or more homology to the amino acid sequence represented by the 255th to 271st amino acids in the amino acid sequence of SEQ ID NO:3;

70% or more homology to the amino acid sequence represented by the 289th to 326th amino acids in the amino acid sequence of SEQ ID NO: 3; or

70% or more homology to the amino acid sequence represented by the 610th to 696th amino acids in the amino acid sequence of SEQ ID NO: 3.

The gene of the present invention from a Cruciferae plant can be confirmed, for example, by the following point:

The amino acid sequence encoded by the nucleotide sequence determined has 75% or more homology to the amino acid sequence represented by the 111th to 213th amino acids in the amino acid sequence of SEQ ID NO: 5;

80% or more homology to the amino acid sequence represented by the 260th to 275th amino acids in the amino acid sequence of SEQ ID NO: 5;

70% or more homology to the amino acid sequence represented by the 293rd to 325th amino acids in the amino acid

sequence of SEQ ID NO: 5; or

70% or more homology to the amino acid sequence represented by the 609th to 695th amino acids in the amino acid sequence of SEQ ID NO: 5.

5           The "homology" used herein means the proportion of the number of amino acids in a region, which are identical to those in a different region to be compared, to the number of the entire amino acids in the former region, upon comparing regions having similarity in two amino acid sequences. In this  
10       respect, it is preferred that the region having similarity contains more amino acids. Such homology of amino acid sequences can be evaluated by using a commercially available gene analysis software such as GENETIX (Software Kaihatu K.K.).

          Further, according to the same manner as described  
15       above, a nucleic acid containing raffinose synthase gene can be detected by hybridization to DNA from the desired organism using the gene fragment as a probe to detect a nucleic acid to which the probe specifically binds (hereinafter referred to as the detection method of the present invention). The gene  
20       fragment used herein can be chemically synthesized according to a conventional method on the basis of the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or 8. Alternatively, it can be prepared by PCR using as primers oligonucleotides chemically synthesized according to a conventional method on the basis of  
25       the nucleotide sequence represented by SEQ ID NO: 2, 4, 6 or

8.

The gene fragment may be a part of the non-translated region of the raffinose synthase gene as well as the open reading frame thereof. For example, an oligonucleotide having the same nucleotide sequence as a part of that of 5'-upstream side such as the 1st to 235th nucleotides in the nucleotide sequence of SEQ ID NO: 4, the 1st to 133rd nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like, or a part of that of 3'-downstream side such as the 2588th to 2675th nucleotides in the nucleotide sequence of SEQ ID NO: 4, the 2468th to 2676th nucleotides in the nucleotide sequence of SEQ ID NO: 6 and the like.

When PCR is carried out by using the gene fragment as primers, it is possible to amplify a nucleic acid containing raffinose synthase gene from DNA derived from the desired organism (hereinafter referred to as the amplification method of the present invention).

More specifically, for example, oligonucleotides having the nucleotide sequences of the gene fragment are designed and chemically synthesized according to a conventional method.

In general, it is preferred that the number of nucleotides is more from a viewpoint that the specificity of annealing is ensured. It is, however, also preferred that the number of nucleotides is not so many from viewpoints that the primers themselves are liable to have a higher structure giving possible

deterioration of the annealing efficiency and that complicated procedures are required in the purification after the synthesis.

Normally, oligonucleotides composed of 15 to 50 bases are preferred. In this respect, based on the codon table showing the correspondence of amino acids encoded by codons, a mixture of primers can also be synthesized by using a mixture of plural bases so that a residue at a specified position in a primer is changed to different bases according to the variation of codons which can encode one certain amino acid. Alternatively, for example, a base such as inosine which can form a base pair with plural bases can be used instead of the above mixture of plural bases.

Coding Table

Phe: UUU, UUC	Ser: UCU, UCC, UCA, UCG, AGU, AGC
Tyr: UAU, UAC	Cys: UGU, UGC
Stop: UAA, UAG, UGA	Trp: UGG
Leu: UUA, UUG, CUU, CUC, CUA, CUG	Pro: CCU, CCC, CCA, CCG
His: CAU, CAC	Gln: CAA, CAG
Arg: CGU, CGC, CGA, CGG, AGA, AGG	
Ile: AUU, AUC, AUA	Thr: ACU, ACC, ACA, ACG
Asn: AAU, AAC	Lys: AAA, AAG
Met: AUG	
Val: GUU, GUC, GUA, GUG	Ala: GCU, GCC, GCA, GCG
Asp: GAU, GAC	Gly: GGU, GGC, GGA, GGG
Glu: GAA, GAG	

In the above codon table, each codon is shown as the nucleotide sequence in mRNA and its light hand is the 5'-terminus. U represents uracil base in RNA and corresponds to thymine base in DNA.

5           An oligonucleotide having the same nucleotide sequence as the coding strand of the double-stranded DNA of the gene of the present invention is called a "sense primer" and that having a nucleotide sequence complementary to the coding strand is called an "antisense primer".

10           A sense primer having the same nucleotide sequence as that of 5'-upstream side in the coding strand of the gene of the present invention, and an antisense primer having a nucleotide sequence complementary to the nucleotide sequence on the 3'-downstream side in this coding strand are used in  
15 combination for PCR reaction, for example, with a gene library, genomic DNA or cDNA as the template to amplify DNA. As the gene library to be used, for example, there are a cDNA library and a genomic library derived from soybean, a Chenopdiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed,  
20 etc. The gene library may also be a library constructed according to a conventional library construction method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor Laboratory Press; "Current Protocols In Molecular Biology" (1987), John Wiley &  
25 Sons, Inc. ISBN 0-471-50338-X, or a commercially available gene



library as such. As the genomic DNA or cDNA, for example, there are those prepared from soybean, a Chenopodiaceae plant such as beet or a Cruciferae plant such as mustard or rapeseed, etc.

For example, PCR is carried out by using the primers 31<sup>(SEQ ID NO: 15)</sup> and 32<sup>(SEQ ID NO: 16)</sup> in the above List 3 and as the template cDNA derived from mustard to amplify DNA having the nucleotide sequence

represented by the 749th to 1215th nucleotides in the nucleotide sequence of SEQ ID NO: 6. Further, PCR is carried out by using the primers and as the template cDNA derived from rapeseed to amplify DNA having the nucleotide sequence represented by the 1st to 467th nucleotides in the nucleotide sequence of SEQ ID NO: 8. The nucleic acid thus amplified can be confirmed by conventional electrophoresis. The nucleic acid can be cloned according a conventional method such as that described in

"Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. For the nucleic acid, its restriction map is prepared or its nucleotide sequence is determined by a conventional method, so that the nucleic acid containing raffinose synthase gene or a part thereof can be identified.

When the nucleic acid contains a part of raffinose synthase, PCR can be carried out on the basis of its nucleotide sequence to amplify the nucleic acid containing the 5'-upstream side nucleotide sequence or the 3'-downstream side nucleotide

sequence. That is, based on the nucleotide sequence of the above-obtained nucleic acid, an antisense primer is designed and synthesized for amplification of the 5'-upstream side part, and a sense primer is designed and synthesized for amplification of the 3'-downstream side part. The nucleotide sequence of the 5'-upstream side part or 3'-downstream side part of the nucleotide sequence already obtained can be determined by the RACE method using these primers and a commercially available kit such as Marathon Kit of Clontech. The full length raffinose synthase gene can be obtained by synthesizing new primers based on both terminal sequences in the nucleotide sequence thus determined and carrying out PCR again.

The above detection method of the present invention can also be used in the analysis of genotypes of a plant such as soybean, a Chenopodiaceae plant or a Cruciferae plant, etc.

More specifically, for example, a genomic DNA derived from soybean, a Chenopodiaceae plant or a Cruciferae plant is prepared according to a conventional method, for example, described in "Cloning and Sequence (Plant Biotechnology Experiment Manual)" compiled under the supervision of Itaru Watanabe, edited by Masahiro Sugiura, published by Noson Bunka-sha, Tokyo (1989).

The genomic DNA is digested with at least several kinds of restriction enzymes, followed by electrophoresis. The electrophoresed DNA is blotted on a filter according to a conventional method. This filter is subjected to hybridization

with a probe prepared from DNA having the gene fragment by a conventional method, and DNA to which the probe hybridizes is detected. The DNAs detected are compared in length between different varieties of a specified plant species. The

5 differences in length make possible the analysis of differences in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides between these varieties.

Furthermore, when the DNAs detected by the above method are compared in length between the gene recombinant plant and the  
10 non-gene recombinant plant of the same variety, the former plant can be distinguished from the latter plant by the detection of hybridizing bands greater in number or higher in concentration for the former plant than for the latter plant. This method can be carried out according to the RFLP (restriction fragment length  
15 polymorphism) method, for example, described in "Plant PCR Experiment Protocols" compiled under the supervision of Ko Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 90-94.

Further, the amplification method of the present  
20 invention can be used for an analysis of genes of soybean, a Chenopdiaceae plant or a Cruciferae plant, etc. More specifically, for example, the amplification method of the present invention is carried out by using plant genomic DNA prepared from soybean, a Chenopdiaceae plant or a Cruciferae  
25 plant to amplify DNA. The amplified DNA is mixed with a

formaldehyde solution, followed by heat denaturing at 85°C for 5 minutes and then quickly cooling on ice. A sample thereof is subjected to electrophoresis on, for example, 6 (w/v)% polyacrylamide gel containing 0 (v/v)% or 10 (v/v)% of glycerol.

5 For this electrophoresis, a commercially available electrophoresis apparatus such as that for SSCP (Single Strand Conformation Polymorphism) can be used and the electrophoresis can be carried out with maintaining the gel at a constant temperature, for example, at 5°C, 25°C, 37°C, etc. From the  
10 electrophoresed gel, DNA is detected, for example, by a method such as silver staining method with a commercially available reagent. From the differences of behavior between the varieties in the electrophoresis of the DNA detected, a mutation in the raffinose synthase gene is detected, and an analysis is carried  
15 out for differences caused by the mutation in phenotypic characteristics accompanied with the expression of raffinose family oligosaccharides. This method can be carried out according to the SSCP method, for example, described in "Plant PCR Experiment Protocols" compiled under the supervision of Ko  
20 Shimamoto and Takuji Sasaki, published by Shujun-sha, Tokyo (1995), ISBN 4-87962-144-7, pp. 141-146.

The analysis of the plant gene from soybean, a Chenopdiaceae plant or a Cruciferae plant by the above detection method or amplification method of the present invention can be  
25 used not only for the analysis of differences in phenotypic

characteristics accompanied with the expression of raffinose family oligosaccharides, but also, for example, for the selection of clones having the desired characters upon production of a novel variety of soybean, a Chenopodiaceae plant or a Cruciferae plant. Further, it can also be used for identification of a clone thus produced and having the characters derived from a recombinant plant upon producing a plant variety using the recombinant plant.

For expression of the gene of the present invention in cells of a host, preferably, a nucleic acid comprising a nucleic acid fragment which contains the gene of the present invention, and a nucleic acid fragment which has a promoter activity in the host cells and joined to the former nucleic acid fragment (hereinafter referred to as the expression nucleic acid of the present invention) can be used.

The nucleic acid fragment having promoter activity in the expression nucleic acid of the present invention is not limited to a specific one, so long as it is functionable in a host to be transformed. For example, there are synthetic promoters functionable in *Escherichia coli*, such as *E. coli* lactose operon promoter, *E. coli* tryptophan operon promoter and tac promoter, etc.; yeast alcohol dehydrogenase gene (ADH) promoter, adenovirus major late (Ad.ML) promoter, SV40 early promoter, baculovirus promoter and the like. When the host is

a plant, the promoter may include, for example, T-DNA derived constitutive promoters such as nopaline synthase gene (NOS) promoter, octopine synthase gene (OCS) promoter, etc.; plant virus-derived promoters such as cauliflower mosaic virus

5 (CaMV)-derived 19S and 35S promoters; inducible promoters such as phenylalanine ammonia-lyase (PAL) gene promoter, chalcone synthase (CHS) gene promoter, pathogenesis-related protein (PR) gene promoter, etc. Furthermore, vector pSUM-GY1 (see JP-A 06-189777/1994) can also be used, which has a promoter giving  
10 specific expression in a specified plant tissue, e.g., a promoter of soybean-derived seed storage protein glycinin gene (JP-A 6-189777).

Furthermore, a nucleic acid fragment having a terminator activity can be joined to the expression nucleic acid  
15 of the present invention. In this case, it is generally preferred that the expression nucleic acid of the present invention is constructed so that the nucleic acid fragment having a terminator activity is positioned downstream the raffinose synthase gene. The terminator to be used is not particularly  
20 limited, so long as it is functionable in cells of a host to be transformed. For example, when the host is a plant, there are T-DNA derived constitutive terminators such as nopaline synthase gene (NOS) terminator, etc.; plant derived terminators such as terminators of allium virus GV1 or GV2, and the like.

25 The expression nucleic acid of the present invention

can be introduced into a host cell according to a conventional gene engineering technique to obtain a transformant. If necessary, the expression nucleic acid of the present invention can be inserted into a vector having a suitable marker depending upon a particular transformation technique for introduction of the nucleic acid into a host cell.

A vector into which the expression nucleic acid of the present invention is inserted can be introduced into a microorganism according to a conventional method, for example, described in "Molecular Cloning: A Laboratory Manual 2nd edition" (1989), Cold Spring Harbor laboratory Press or "Current Protocols in Molecular Biology" (1987), John Wiley & Sons, Inc., ISBN 0-471-50338-X. The microorganism transformed with the vector can be selected on the basis of a selection marker such as antibiotic resistance, auxotrophy or the like. In case that the gene of the present invention is joined to the downstream of an inducible promoter (e.g., tac promoter) in the translatable form in the selected microorganism (e.g., *E. coli* transformant), a translated product of the gene of the present invention can be expressed under conventional culture and inducible conditions and can be recovered as a peptide or a protein.

The raffinose synthase activity of the translated product of the gene of the present invention thus prepared can be measured by, for example, a method described in L. Lehle and W. Tanner, Eur. J. Biochem., 38, 103-110 (1973) to identify the

translated product having the "capability of binding D-galactosyl group through  $\alpha$  (1 $\rightarrow$ 6) bond to the hydroxyl group attached to the carbon atom at 6-position of the D-glucose residue in sucrose molecule". More specifically, for example,

5 the gene of the present invention is cloned in pGEX-4T3 (Pharmacia) to obtain a plasmid containing the expression nucleic acid of the present invention. The resultant plasmid is introduced into, for example, *E. coli* HB101 strain to obtain a transformant. The resultant transformant is culture

10 overnight and 1 ml of the culture is inoculated into 100 ml of LB culture medium. It is incubated at 37°C for about 3 hours and IPTG (isopropylthio- $\beta$ -D-galactoside) is added at a final concentration of 1 mM, followed by further incubation for 5 hours.

Cells are recovered from the culture broth by centrifugation

15 and are suspended by addition of 10 times of the cell weight of 100 mM Tris-HCl (pH 7.4), 1 mM EDTA, 5 mM DTT (dithiothreitol), 1 mM PMSF (phenylmethylsulfonyl fluoride) and 1 mM benzamide.

The suspension is sonicated with a ultrasonic disrupter (Branson) to disrupt the cells. The disrupted cell suspension

20 is centrifuged to recover a soluble protein solution. The resultant protein solution is added to a reaction mixture containing at final concentrations of 100 mM Tris-HCl (pH 7.4), 5 mM DTT (dithiothreitol), 0.01% BSA, 200  $\mu$ M sucrose, 5 mM galactinol and 31.7  $\mu$ M [ $^{14}$ C] sucrose. The reaction mixture is

25 incubated at 37°C, followed by addition of 1.5 times in volume



of ethanol and stirring. Insoluble materials are removed by centrifugation, the supernatant is spotted on, for example, a HPTLC cellulose thin layer chromatography plate (Merck, HPTLC plates cellulose) and then the plate is developed with n-butanol-pyridine-water-acetic acid (60 : 40 : 30 : 3). The developed plate is dried and analyzed with an imaging analyzer (FUJIX Bio-Image Analyzer BAS-2000II manufactured by Fuji Film) to determine [<sup>14</sup>C] raffinose produced to measure the raffinose synthase activity.

In addition, the translated product as prepared above can also be used as an antigen for producing an antibody. The antibody thus produced can be used for, for example, detection and determination of the gene of the present invention in a crude protein extract prepared from an organism such as a plant.

When the host is a plant, the vector into which the gene of the present invention is inserted can be introduced into plant cells by a conventional means such as *Agrobacterium* infection method (JP-B 2-58917 and JP-A 60-70080), electroporation into protoplasts (JP-A 60-251887 and JP-B 5-68575) or particle gun method (JP-A 5-508316 and JP-A 63-258525). The plant cell transformed by the introduction of the vector can be selected on the basis of a selection marker, for example, resistance to an antibiotic such as kanamycin or hygromycin. From the plant cell thus transformed, a transformed plant can be regenerated by a conventional plant cell cultivation

method, for example, described in "Plant Gene Manipulation Manual (How to Produce Transgenic Plants)" written by Uchimiya, 1990, Kodan-sha Scientific (ISBN 4-06-153513-7), pp. 27-55.

Furthermore, the collection of seeds from the transformed plant  
5 also makes it possible to proliferate the transformed plant. In addition, crossing between the transformed plant obtained and the non-transformed plant makes it possible to produce progenic plants with the characters of the transformed plant.

As gene engineering techniques in soybean, basically,  
10 the above general techniques can be employed. More specifically, "transformation of soybean plant strain by particle gun" described in EP 301749, gene introduction methods. for example, described in Torisky, R.S., Kovacs, L., Avdiushko, S., Newman, J.D., Hunt, A.G. and Collins, G.B., "Development of a binary  
15 vector system for plant transformation based on the supervirulent *Agrobacterium tumefaciens* strain Chry5", Plant Cell Rep., (1997), 17, p. 102-108, etc. can be employed.

As gene engineering techniques in a *Chenopodiaceae* plant, basically, the above general techniques can be employed.  
20 More specifically, gene introduction methods, for example, described in M. Mannerlof, S. Tuveesson, P. Steen and P. Tenning, "Transgenic sugar beet tolerant to glyphosate", Euphytica (1997), 94, p 83-91, B.K. Konwar, "Agrobacterium tumefaciens-Mediated Genetic Transformation of Sugar Beet  
25 (Beta vulgaris L.)", J. Plant Biochemistry & Biotechnology

(1994), 3, p. 37-41 can be employed.

As gene engineering techniques in a Cruciferae plant, basically, the above general techniques can be employed. More specifically, the gene introduction can be carried out according to a method, for example, described in J. Fry, A. Barnason and R.B. Horsch, "Transformation of Brassica napus with *Agrobacterium tumefaciens* based vectors", Plant Cell Reports (1987), 6, 321-325.

For example, when gene introduction is carried out by *Agrobacterium* infection method, first, the above-described expression nucleic acid of the present invention is inserted into a binary vector. The resultant vector can be introduced into, for example, *Agrobacterium tumefaciens* LBA 4404 strain which has been converted into a competent state by treatment with calcium chloride. A transformant can be selected by an appropriate selection method according to the selection marker gene of the vector, for example, cultivation of a strain containing the vector in a culture medium containing an antibiotic in case that the selection marker gene is that giving resistance to the antibiotic such as kanamycin. The resultant transformed *Agrobacterium* strain can be culture in a liquid culture medium, for example, LB medium.

Soybean, a Chenopdiaceae plant or a Cruciferae plant can be transformed by using thus obtained *Agrobacterium*

transformant culture broth as described below. For example, seeds from soybean, beet, rapeseed or mustard is sowed aseptically in, for example, 1/2 MS medium containing 2% sucrose and 0.7% agar. After about 1 week, cotyledons and petioles of the germinated plant are cut off with a scalpel aseptically and transplanted in, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5  $\mu\text{M}$  BA, 0.05  $\mu\text{M}$  2,4-D and 3.3  $\mu\text{M}$   $\text{AgNO}_3$  and cultured for one day. The cotyledons and petioles thus precultured are transferred to 1000-fold dilution of the above *Agrobacterium* culture broth and allowed to stand for 5 minutes. The cotyledons and petioles are transferred to the same medium as that of the preculture again and cultured for about 3 to 4 days. The cotyledones and petioles thus cultured are transferred to, for example, MS medium containing 3% sucrose, 4.5  $\mu\text{M}$  BA, 0.05  $\mu\text{M}$  2,4-D, 3.3  $\mu\text{M}$   $\text{AgNO}_3$  and 500 mg/liter cefotaxim, followed by shaking for 1 day to remove microbial cells. The resultant cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5  $\mu\text{M}$  BA, 0.05  $\mu\text{M}$  2,4-D, 3.3  $\mu\text{M}$   $\text{AgNO}_3$  100 mg/liter cefotaxim and 20 mg/liter kanamycin, followed by culturing for 3 to 4 weeks. Then, the cotyledons and petioles are transferred to, for example, MS medium containing 3% sucrose, 0.7% agar, 4.5  $\mu\text{M}$  BA, 0.05  $\mu\text{M}$  2,4-D, 100 mg/liter cefotaxim and 20 mg/liter kanamycin and cultured. Culture in this medium is continued with subculturing every 3 to 4 weeks. When a shoot are regenerated, it is subcultured in,

for example, MS medium containing 3% sucrose, 0.7% agar and 20 mg/liter kanamycin for 3 to 4 weeks. When the plant makes roots, it is transferred to vermiculite-peat moss (1 : 1) and acclimatized by culturing at 21 to 22°C under day and night conditions of 12 hours: 12 hours = day time : night. As the plant grows, it is transferred to appropriate cultivation soil to culture the plant. A genomic DNA is extracted from the leaf of the regenerated plant according to the above method and PCR is carried out by using as primers having partial nucleotide sequences of the expression nucleic acid of the present invention to confirm the insertion of the gene of the present invention into the plant.

As described hereinabove, by introducing the gene of the present invention into a plant, for example, soybean, a Chenopdiaceae plant or a Cruciferae plant, it is possible to vary the expression level and activity of raffinose synthase in the plant to control the amount of raffinose family oligosaccharides in the plant. The gene of the present invention is useful in techniques for varying the expression level and activity of raffinose synthase in soybean, a Chenopdiaceae plant or a Cruciferae plant on the basis of gene homology, for example, techniques such as homologous recombination and antisense technique, cosuppression and the like.

The following examples further illustrate the present

invention in detail but are not to be construed to limit the scope of the present invention.

Example 1

Preparation of cDNA Derived from Soybean

5           About 2 g of immature seeds of soybean (*Glycine max*) Williams82 were frozen in liquid nitrogen and then ground with a mortar, to which 20 ml of Isogen (Nippon Gene) was added, and the mixture was further thoroughly ground. The ground material was transferred into a centrifugation tube, to which 4 ml of  
10 chloroform was added, and the mixture was stirred with a vortex mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer was collected, to which 10 ml of isopropanol was added, and the mixture was stirred and then centrifuged at 6,500 x g for 10 minutes at 4°C. The resulting precipitate was washed  
15 with 10 ml of 70% ethanol and then dissolved in 1 ml of elution buffer (10 mM Tris-HCl/pH 7.5, 1 mM EDTA, 0.1% SDS). The solution was allowed to stand at 60°C for 10 minutes and then centrifuged at 10,000 x g for 1 minute to remove insoluble matter. To the resulting supernatant was added an equivalent volume of  
20 Oligotex-dT30 (Takara), and the mixture was stirred and then allowed to stand at 65°C for 5 minutes. Further, the mixture was placed on ice and allowed to stand for 3 minutes, to which 200 µl of 5 M NaCl was added, and the mixture was mixed and then allowed to stand at 37°C for 10 minutes. The mixture was then centrifuged

at 10,000 x g for 3 minutes at 4°C. The precipitate was collected and then suspended in 1 ml of TE buffer, and the suspension was allowed to stand at 65°C for 5 minutes. Further, the suspension was placed on ice and then allowing to stand for 3 minutes, followed  
5 by centrifugation at 10,000 x g for 3 minutes at 4°C to remove precipitate.

To the resulting supernatant were added 100 µl of 3M sodium acetate and 2 ml of ethanol to precipitate and collect RNA.

The collected RNA was washed twice with 70% ethanol and then  
10 dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, First Strand Synthesis Kit for RT-PCR (Amersham) and cDNA Synthesis Kit (Takara) were used, and  
15 all operations were made according to the protocol attached to kits.

#### Example 2

Cloning of Raffinose Synthase Gene from Soybean cDNA

PCR was carried out by using the cDNA obtained from  
20 immature seeds of soybean (*Glycine max*) Williams82 in Example 1 as a template and the primers designed on the basis of the amino acid sequence of SEQ ID No: 1, i.e., primers having nucleotide  
(SEQ ID Nos: 21+22)  
sequences shown in List 4 below to amplify a DNA fragment. The PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal

Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The reaction was carried out by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times to amplify the DNA fragment.

5 The amplified DNA fragment was cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of the nucleotide sequence with a 373S DNA sequencer of ABI. Based on this sequence, primers having nucleotide  
10 sequences shown in List 5 <sup>(SEQ ID NO:23)</sup> below were synthesized. The synthesis of cDNA was carried out with Marathon Kit of Clontech using mRNA obtained from leaves of soybean Williams82 in Example 1. The cDNA obtained was ligated to an adapter contained in the kit with ligase. These operations were carried out according to the protocol  
15 attached to the kit. By using the adapter-ligated cDNA thus prepared, PCR was carried out with the primers shown in List 5 <sup>(SEQ ID NO:24)</sup> according to the same manner as the above. The nucleotide sequence in terminal region of the gene was analyzed according to the protocol attached to the Marathon Kit of Clontech. As a result,  
20 the nucleotide sequence of SEQ ID NO: 2 was determined.

List 4

4-5-F primer: <sup>(SEQ ID NO:21)</sup>

cgatggatgg giaaittiat icaiccgai tgggaiatgt t 41mer

4-6-RV primer: <sup>(SEQ ID NO:22)</sup>



ggccacatit tiacia(ag)icc iatiggigci aa 32mer

List 5

5-SC-2: (SEQ ID NO: 23)

tggtactagg cgaaacaaga gtagctctga 30mer

5 Example 3

Preparation of cDNA derived from Chenopdiaceae Plant

10 About 2 g of leaves of beet (*Beta vulgaris*: haming)  
was frozen in liquid nitrogen and then ground with a mortar,  
to which 20 ml of Isogen (Nippon Gene) was added, and the mixture  
was further thoroughly ground. The ground material was  
transferred into a centrifugation tube, to which 4 ml of  
chloroform was added, and the mixture was stirred with a vortex  
mixer and then centrifuged at 6,500 x g for 10 minutes at 4°C.  
15 The water layer was collected, to which 10 ml of isopropanol was  
added, and the mixture was stirred and then centrifuged at 6,500  
x g for 10 minutes at 4°C. The resulting precipitate was washed  
with 10 ml of 70% ethanol and then dissolved in 180 µl of  
DEPC-treated sterilized water. The solution was allowed to stand  
at 55°C for 5 minutes and 20 µl of 5 M NaCl was added thereto. The  
20 resulting solution was purified using BIOMAG mRNA PURIFICATION  
KIT (PerSeptive Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium  
acetate and ethanol, and RNA was precipitated and collected. The  
collected RNA was washed twice with 70% ethanol and then dissolved

in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

5 For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were made according to the protocol attached to the kit.

#### Example 4

Analysis of Nucleotide Sequence of Raffinose Synthase Gene from Chenopdiaceae Plant

10 Synthetic DNA primers having the nucleotide sequences shown in List 6 below were synthesized. The PCR method was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cyclor Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The PCR was carried out with the above primers and cDNA of beet  
15 obtained in the above Example 3 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. As a result, the combinations of primers 6-3-F and 6-8-RV and primers 6-10-F and 6-6-RV gave an amplification of about 0.3 kb and 0.6 kb bands, respectively. The  
20 amplified DNA fragments were cloned with TA cloning kit (Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and analysis of nucleotide sequence with a 373S DNA sequencer of ABI. Based on the resulting nucleotide sequences, synthetic DNA

(SEQ ID NOS: 28-35)  
primers having nucleotide sequences shown in List 7 below were prepared and PCR was carried out using cDNA obtained from beet in Example 3 in the same manner as above. As a result, DNA having the nucleotide sequence of SEQ ID NO: 4 was finally obtained from

5 cDNA of beet.

List 6

6-3-F: (SEQ ID NO: 24)

cgaggigggt gicciccigg ittigtati atigaigaig gitggca 47mer

6-8-RV: (SEQ ID NO: 25)

10 at(t/c)tt(a/g)tcia cigcia(a/g)(a/g)tc (t/c)tccatigt 29mer

6-10-F: (SEQ ID NO: 26)

ggiacitait gg(c/t)ticaigg itgicaiatg gticaigt 38mer

6-6-RV: (SEQ ID NO: 27)

ggccacatit tiacia(a/g)icc iatiggigci aa 32mer

15 List 7

7-Sb-1: (SEQ ID NO: 28)

atctatttgt catgacgatg atccga 26mer

7-Sb-2RV: (SEQ ID NO: 29)

ggccctcatt cccatattgg gatgatcctc 30mer

20 7-Sb-3RV: (SEQ ID NO: 30)

aagcatgccaa aacatacaca tgctcaacag 30mer

7-Sb-4RV: (SEQ ID NO: 31)

agacccgggg aaagctttgg ggttactact 30mer

7-Sb-5: (SEQ ID NO: 32)

25 tggatgggaa actttataca ccctgact 28mer

7-Sb-6: (SEQ ID NO:33)

gacatgttcc catctacaca cccttggtg 28mer

7-Sb-7: (SEQ ID NO:34)

ccaatttatg ttagtgatgt tggtggcaag 30mer

5 7-Sb-8RV: (SEQ ID NO:35)

tcgactccca gggtagaatt gtcac 26mer

#### Example 5

##### Preparation of cDNA Derived from Cruciferae Plant

About 2 g of leaves of mustard (*Brassica juncea*) was  
10 frozen in liquid nitrogen and then ground with a mortar, to which  
20 ml of Isogen (Nippon Gene) was added, and the mixture was  
further thoroughly ground. The ground material was transferred  
into a centrifugation tube, to which 4 ml of chloroform was added,  
and the mixture was stirred with a vortex mixer and then  
15 centrifuged at 6,500 x g for 10 minutes at 4°C. The water layer  
was collected, to which 10 ml of isopropanol was added, and the  
mixture was stirred and then centrifuged at 6,500 x g for 10 minutes  
at 4°C. The resulting precipitate was washed with 10 ml of 70%  
ethanol and then dissolved in 180 µl of DEPC-treated sterilized  
20 water. The solution was allowed to stand at 55°C for 5 minutes  
and to which 20 µl of 5 M NaCl was added. The resulting solution  
was purified using BIOMAG mRNA PURIFICATION KIT (PerSeptive  
Biosystems: Catalog No. 8-MB4003K).

To the resulting mRNA solution were added 3M sodium

acetate and ethanol, and RNA was precipitated and collected. The collected RNA was washed twice with 70% ethanol and then dissolved in 20 µl of sterilized water, which was used for the subsequent cDNA synthesis. The amount of RNA obtained was determined by the measurement of absorbance at 260 nm.

For the cDNA synthesis, SMART PCR cDNA Synthesis Kit (Clontech) was used, and all operations were carried out according to the protocol attached to the kit.

In the same manner as described in the above, mRNA was purified from immature seeds of rapeseed Westar (*Brassica napus*) and cDNA was synthesized.

#### Example 6

Isolation and Nucleotide Sequence Analysis of  
Raffinose Synthase Gene derived from Cruciferae Plant

DNA primers having the nucleotide sequences shown in List 8 below were synthesized. PCR was carried out with Gene Amp PCR Systems 2400 and DNA Thermal Cycler Model 480 of Perkin-Elmer using Advantage KlenTaq cDNA Kit of Clontech. The PCR was carried out with the above primers and cDNA of mustard obtained in Example 5 by repeating the cycle for maintaining at 94°C for 1 minute, at 50°C for 3 minutes and then at 72°C for 3 minutes 40 times. The reaction products were analyzed by agarose gel electrophoresis. As a result, an amplification of about the 1.2 kb bands was detected. The amplified DNA fragment was cloned with TA cloning kit

(Invitrogen), followed by sequence reaction using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit of Perkin-Elmer and nucleotide sequence analysis with a 373S DNA sequencer of ABI.

Based on the resulting nucleotide sequence, synthetic primers  
 5 having the nucleotide sequences shown in List 9 below were prepared  
 and PCR was carried out using cDNAs from mustard (*Brassica juncea*)  
 and rapeseed Westar (*Brassica napus*) obtained in Example 5  
 according to the same manner as the above. As a result, the  
 nucleotide sequence represented by the 749th to 1215th nucleotides  
 10 of SEQ ID NO: 6 and by the 1st to 467th nucleotides of SEQ ID NO:  
 8 were finally determined for cDNA from mustard (*Brassica juncea*)  
 and rapeseed Westar (*Brassica napus*), respectively.

#### List 8

8-#1: (SEQ ID NO: 36)  
 15 cgattiaaig titggtggac iacicaityg gtigg 35mer  
 8-#10RV: (SEQ ID NO: 37)  
 caitgiacca titgicaicc itgia(ag)ccai taigticc 38mer

#### List 9

9-primer-1: (SEQ ID NO: 38)  
 20 gttagggttc atatgaacac cttcaagctc 30mer  
 9-primer-2RV: (SEQ ID NO: 39)  
 caacggcgag atcttgcac gtcaac 26mer

#### Example 7

#### Nucleotide Sequence Analysis of Raffinose Synthase

# Full-Length Gene Derived from Cruciferae Plant

Based on the nucleotide sequences obtained in Example  
(SEQ ID NOS: 6, 8, 39-39)  
6, DNA primers having the nucleotide sequences shown in List  
(SEQ ID NOS: 40-46)  
10 below were synthesized. The cDNAs from mustard (*Brassica*  
5 *juncea*) and rapeseed Westar (*Brassica napus*) obtained in the same  
manner as described in Example 5 were ligated to adapters  
contained in Marathon Kit of Clontech. By using the adapter-  
ligated cDNAs thus prepared, PCR was carried out with primers shown  
in List 10. 10-B-2RV, 10-B-3RV and 10-B-4RV primers were used for  
(SEQ ID NO: 40) (SEQ ID NO: 41)  
10 nucleotide analysis of 5'-termini, and 10-B-1, 10-B-8, 10-B-7 and  
(SEQ ID NO: 43) (SEQ ID NO: 45)  
(SEQ ID NO: 46)  
10-B-6 primers were used for nucleotide analysis of 3'-termini.  
The nucleotide sequences were analyzed according to the protocol  
attached to the Marathon Kit of clontech. As a result, the  
nucleotide sequence of SEQ ID NO: 6 and SEQ ID NO: 8 were determined  
15 from mustard (*Brassica juncea*) and rapeseed Westar (*Brassica*  
*napus*), respectively.

## List 10

10-B-2RV: (SEQ ID NO: 40)  
ggattcgaca caaaccgcca cgtcacgctc 30mer  
20 10-B-3RV: (SEQ ID NO: 41)  
ccacgtgcac caccggaact tatcgac 27mer  
10-B-4RV: (SEQ ID NO: 42)  
aacatcgata ccatcggagt catgtccaat 30mer  
10-B-1: (SEQ ID NO: 43)

gtaggggttc atatgaacac cttcaagctc 30mer

10-B-8: (SEQ ID NO: 44)

tctacgtctg gcacgcgctt tgcggctac 29mer

10-B-7: (SEQ ID NO: 45)

5 gttgacgtca tccacatatt ggagatgttg t 31mer

10-B-6: (SEQ ID NO: 46)

gttatcgcta gcatggagca ctgtaatga 29mer

#### Example 8

### 10 Construction of Expression Vectors in Plant for Raffinose Synthase Gene Derived from Cruciferae Plant

Based on the nucleotide sequence of raffinose synthase gene from mustard obtained in Example 7, DNA primers having the nucleotide sequences shown in List 11 (SEQ ID NOS: 47-48) were prepared. By using cDNA of mustard, PCR was carried out in the same manner as described in Example 6. The amplified DNA fragment was digested with SacI. The DNA fragment thus digested was ligated to the vector pBI121(-) previously digested with SacI by using Ligation Kit (Takara). Plasmid pBI121 (Clontech) were digested with BamHI and SacI, and ligated to linkers shown in List 12 (SEQ ID NOS: 49-50) to prepare

20 the vector pBI121(-). The vector thus obtained was analyzed by a restriction map and PCR using primers having nucleotide sequences shown in List 13, (SEQ ID NOS: 51-53) and confirmed the direction of inserted raffinose synthase gene. The vector whose raffinose synthase gene from mustard was inserted in the expressible

25 direction was designated BjRS-Sac(+)-121 and the one whose



raffinose synthase gene from mustard was inserted in the reverse direction was designated BjRS-Sac(-)-121.

List 11

11-SacI-BjN:(SEQ ID NO:47)

5 aacgagctca atccaaaatc tcatcaaata atcgc 35mer

11-SacI-BjintRV:(SEQ ID NO:48)

acaatagttg agggcggaag agtag 25mer

List 12

12-BamSac-(+)linker:(SEQ ID NO:49)

10 gatcgagctc gtgtcggatc cagct 25mer

12-BamSac-(-)linker:(SEQ ID NO:50)

ggatccgaca cgagctc 17mer

List 13

13-35S-3:(SEQ ID NO:51)

15 cctcctcgga ttccattgcc cagctatctg 30mer

13-B-2RV:(SEQ ID NO:52)

ggattcgaca caaaccgcca cgtcatcgtc 30mer

13-B-8:(SEQ ID NO:53)

tctacgtctg gcacgcgctt tgcggctac 29mer

20 Example 9

Transformation with Raffinose Synthase Gene Derived from Cruciferae Plant

The vectors BjRS-Sac(+)-121 and BjRS-Sac(-)-121 prepared in Example 8 were used for the transformation of mustard  
25 (*Brassica juncea*) by the *Agrobacterium* infection method.

*Agrobacterium tumefaciens* (strain LBA4404 having rifampicin and streptomycin resistance) previously converted into a competent state by calcium chloride treatment was transformed independently with two plasmids BjRS-Sac(+)-121 and BjRS-Sac(-)-121 prepared in Example 8. The transformants were selected on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin by utilizing the kanamycin resistant character conferred by the kanamycin resistant gene (neomycin phosphotransferase, NPTII) of the introduced plasmids.

The transformant *Agrobacterium* obtained (*Agrobacterium tumefaciens* strain LBA4404: rifampicin and streptomycin resistant) was cultured on LB medium containing 50 µg/ml rifampicin and 25 µg/ml kanamycin at 28°C for a whole day and night, and the culture was used for the transformation of mustard by the method described below.

The seeds of mustard were aseptically sowed on 1/2 MS medium containing 2% sucrose and 0.7% agar. After one week, cotyledons and petioles of sprouting plants were cut out with a scalpel, and transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5 µM BA, 0.05 µM 2,4-D and 3.3 µM AgNO<sub>3</sub>, followed by preculture for 1 day. The precultured cotyledons and petioles were transferred in a 1000-fold dilution of the *Agrobacterium* culture broth and allowed to stand for 5 minutes. The cotyledons and petioles were transferred again to the same

medium as used in the preculture, and cultured for 3 to 4 days.

The cultured cotyledons and petioles were transferred to MS medium containing 3% sucrose, 4.5  $\mu$ M BA, 0.05  $\mu$ M 2.4-D, 3.3  $\mu$ M AgNO<sub>3</sub> and 500 mg/l cefotaxim, and shaken for 1 day to remove microbial cells. The cotyledons and petioles thus treated were transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5  $\mu$ M BA, 0.05  $\mu$ M 2.4-D, 3.3  $\mu$ M AgNO<sub>3</sub>, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultured for 3 to 4 weeks. The cotyledons and petioles were transferred to MS medium containing 3% sucrose, 0.7% agar, 4.5  $\mu$ M BA, 0.05  $\mu$ M 2.4-D, 100 mg/l cefotaxim and 20 mg/l kanamycin, and cultivated. The cultivation on this medium was continued with subculturing at intervals of 3 to 4 weeks.

When shoots are began to regenerate, these shoots are subcultured on MS medium containing 3% sucrose, 0.7% agar and 20 mg/l kanamycin, and cultivated for 3 to 4 weeks. The rooting plants are transferred to vermiculite : peat moss = 1 : 1, and cultivated at 21°C to 22°C in a cycle of day/night = 12 hours : 12 hours. With the progress of plant body growth, the plants are grown with cultivation soil.

20

#### BRIEF DESCRIPTION OF SEQUENCES

SEQ ID NO: 1 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene of the present invention.

SEQ ID NO: 2 shows a nucleotide sequence of the raffinose synthase gene of the present invention.

5 SEQ ID NO: 3 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from beet.

SEQ ID NO: 4 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from beet.

10 SEQ ID NO: 5 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from mustard.

SEQ ID NO: 6 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from mustard.

15 SEQ ID NO: 7 shows an amino acid sequence of a raffinose synthase protein encoded by the raffinose synthase gene obtained from rapeseed.

SEQ ID NO: 8 shows a cDNA nucleotide sequence of the raffinose synthase gene obtained from rapeseed.

List 1: (SEQ ID NOS: 9+10)

20 The nucleotide sequences shown in List 1 are examples of the typical primers used in the amplification of a DNA fragment having a raffinose synthase gene. All of these sequences are based on the nucleotide sequence of SEQ ID NO: 2. Primer 11 is a sense primer and Primer 12 is an antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes  
25 can be added to the 5'-termini of these nucleotide sequences.

List 2:

The nucleotide sequences shown in List 2 are examples (SEQ ID NO: 11-14) of the typical primers used in the amplification of a cDNA of a raffinose synthase gene. Primer 21 is a sense primer (SEQ ID NO: 11) corresponding to the 5'-terminus of the beet-derived raffinose synthase gene. Primer 22 is an antisense primer corresponding to the 3'-terminus. (SEQ ID NO: 12) Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

10 Primer 23 is a sense primer corresponding to the N-terminus of the open reading frame, and primer 24 is an antisense primer corresponding to the C-terminus. (SEQ ID NO: 13) (SEQ ID NO: 14)

List 3:

15 Among the nucleotide sequences shown in List 3, primers 31 and 32 are typical primers used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene. Primer 31 is a sense primer used in the amplification of a DNA having the partial nucleotide sequence of a raffinose synthase gene from mustard and rapeseed and primer 32 is an (SEQ ID NO: 15) (SEQ ID NO: 16) antisense primer. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

Primers 33 and 34 are the typical primers used in the amplification of a cDNA of a raffinose synthase gene of mustard. (SEQ ID NO: 17) (SEQ ID NO: 18)

25 Primers 33 and 34 are both based on the nucleotide sequence of (SEQ ID NO: 17) (SEQ ID NO: 18)

raffinose synthase gene in the non-translated region. Primer 33<sup>(SEQ ID NO:17)</sup> is a sense primer corresponding to the 5'-terminal non-translated region of the mustard-derived raffinose synthase gene. Primer 34<sup>(SEQ ID NO:18)</sup> is an antisense primer corresponding to the 3'-terminal non-

5 translated region.

Primers 35 and 36 are typical primers used in the amplification of an open reading frame coding for the amino acid sequence of a raffinose synthase protein in the cDNA of a raffinose synthase gene. Primer 35<sup>(SEQ ID NO:19)</sup> is a sense primer corresponding to the 5'-terminus of the above open reading frame. Primer 36<sup>(SEQ ID NO:20)</sup> is an antisense primer corresponding to the 3'-terminus. Depending upon the purpose, recognition sequences for suitable restriction enzymes can be added to the 5'-termini of these nucleotide sequences.

10

15 List 4: (SEQ ID NOS:21+22)

The nucleotide sequences shown in List 4 are of the primers used in the cloning of a DNA fragment having the present raffinose synthase gene. The base represented by the symbol "i" is inosine. The bases shown in parentheses mean that a mixture of those bases is used in the synthesis. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

20

List 5: (SEQ ID NO:23)

The nucleotide sequence shown in List 5 is of the primer used in the analysis of a nucleotide sequence of the present

25

raffinose synthase gene. 5-SC-2 <sup>(SEQ ID NO: 23)</sup> is used in the analysis of the present nucleotide sequence in the 3'-terminal region.

List 6

The nucleotide sequences shown in List 6 <sup>(SEQ ID NOS: 24-27)</sup> are of the  
5 primers used in the analysis of the present raffinose synthase  
gene of beet. The base represented by the symbol "i" is inosine.  
The bases shown in parentheses mean that a mixture of those bases  
was used in the synthesis. The symbol "RV" as used after the primer  
number means that the primer referred to by this symbol has an  
10 antisense sequence.

List 7 <sup>(SEQ ID NOS: 28-35)</sup>

The nucleotide sequences shown in List 7 are of the  
primers synthesized on the partial nucleotide sequences of the  
beet raffinose synthase gene. The symbol "RV" as used after the  
15 primer number means that the primer referred to by this symbol  
has an antisense sequence.

List 8: <sup>(SEQ ID NOS: 36+37)</sup>

The nucleotide sequences shown in List 8 are of the  
primers used in the analysis of the cDNA nucleotide sequence of  
20 a raffinose synthase gene of mustard. The base represented by the  
symbol "i" is inosine. The bases shown in parentheses mean that  
a mixture of those bases. The symbol "RV" as used after the primer  
number means that the primer referred to by this symbol has an  
antisense sequence.

25 List 9:

The nucleotide sequences shown in List 9 are of the primers synthesized on the partial nucleotide sequences of the mustard raffinose synthase gene. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 10: (SEQ ID NOS: 40-46)

The nucleotide sequences shown in List 10 are of the primers used in the analysis of the nucleotide sequences of raffinose synthase gene of mustard and rapeseed. The symbol "RV" as used after the primer number means that the primer referred to by this symbol has an antisense sequence.

List 11: (SEQ ID NOS 47+48)

The nucleotide sequences shown in List 11 are of the primers used in the amplification of 5'-terminal region of a mustard raffinose synthase gene. 11-SacI-BjN is a primer whose SacI restriction site is added to the nucleotide sequence represented by the 4th to 29th nucleotides in SEQ ID NO: 6.

11-SacI-BjintRV is an antisense primer having a nucleotide sequence corresponding to the nucleotide sequence represented by the 1164th to 1188th nucleotides in SEQ ID NO: 6.

List 12: (SEQ ID NOS: 49+50)

The nucleotide sequences shown in List 12 are of the adapters added to a mustard cDNA. These synthetic DNA take a double-stranded form when mixed together because they are complementary strands. This adapter has cohesive ends of cleavage



sites for the restriction enzymes BamHI and SacI on both termini, and contains the restriction sites for the restriction enzymes BamHI and SacI in the double-stranded region.

List 13: (SEQ ID NOS: 51-53)

5 The nucleotide sequences shown in List 13 are of the primers used in the confirmation of inserting direction of the mustard-derived raffinose synthase gene. 13-35S-3 is a primer of (SEQ ID NO: 51) sense to 35S promoter. 13-B-2RV is an antisense primer having the (SEQ ID NO: 52) nucleotide sequence represented by the 593rd to 622nd nucleotides of SEQ ID NO: 6, 13-B-8 is a sense primer having the nucleotide (SEQ ID NO: 53) sequence represented by the 1110th to 1138th nucleotides in SEQ ID NO: 6.

10

As described hereinabove, according to the present invention, it is possible to provide raffinose synthase genes which can be utilized in techniques for varying expression level and activity of raffinose synthase in plants.

15

#### SEQUENCE LISTING FREE TEXT

20 SEQ ID NO: 9 to SEQ ID NO: 20: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 21 and SEQ ID NO: 22: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

25 SEQ ID NO: 23: Designated oligonucleotide primer to

obtain raffinose synthase gene.

SEQ ID NO: 24 to SEQ ID NO: 27: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, y is t or c, r is a or g.

5 SEQ ID NO: 28 to SEQ ID NO: 35: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 36 and SEQ ID NO: 37: Designed oligonucleotide primer to obtain raffinose synthase gene, n is i, r is a or g.

10 SEQ ID NO: 38 to SEQ ID NO: 48: Designed oligonucleotide primer to obtain raffinose synthase gene.

SEQ ID NO: 49 and SEQ ID NO: 50: Designed oligonucleotide linker to obtain raffinose synthase gene.

15 SEQ ID NO: 51 to SEQ ID NO: 53: Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

SEQUENCE LISTING

5 <110> Eijiro WATANABE et al.; Sumitomo Chemical Company Limited

<120> Raffinose Synthase Genes and Their Use

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10 <151> 1998-04-30

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35 40 45

35 Gln His Tyr Ala Leu Pro Thr Arg Asp Cys Leu Phe Val Asp Pro Leu

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	Gln Asn Val Gly Ile Asp Gly Val Lys Val Asp Val Ile His Ile Leu							
		400			405		410	
	gag atg ttg tgc gag aaa tat ggc ggg aga gta gac ttg gct aaa gct							1417
	Glu Met Leu Cys Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala							
10		415			420		425	
	tac ttc aag gcg tta act tcc tca gtg aat aag cat ttt gac ggt aac							1465
	Tyr Phe Lys Ala Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn							
		430			435		440	
	ggc gtt atc gct agc atg gag cac tgt aat gat ttc atg ttc ctt gga							1513
15	Gly Val Ile Ala Ser Met Glu His Cys Asn Asp Phe Met Phe Leu Gly							
		445			450		455	460
	acc gaa gcc atc tct cta ggt cgt gtc ggt gat gac ttt tgg tgc acg							1561
	Thr Glu Ala Ile Ser Leu Gly Arg Val Gly Asp Asp Phe Trp Cys Thr							
		465			470		475	
20	gat cca tca ggc gac ata aac ggc aca tat tgg ctg caa gga tgc cac							1609
	Asp Pro Ser Gly Asp Ile Asn Gly Thr Tyr Trp Leu Gln Gly Cys His							
		480			485		490	
	atg gtc cac tgt gcc tac aac agt ctt tgg atg gga aat ttc atc cag							1657
	Met Val His Cys Ala Tyr Asn Ser Leu Trp Met Gly Asn Phe Ile Gln							
25		495			500		505	
	cct gat tgg gac atg ttt cag tcc aca cat cct tgt gct gag ttc cat							1705
	Pro Asp Trp Asp Met Phe Gln Ser Thr His Pro Cys Ala Glu Phe His							
		510			515		520	
	gct gct tct cgt gcc atc tcc ggt ggg ccc att tac atc agc gat tgt							1753
30	Ala Ala Ser Arg Ala Ile Ser Gly Gly Pro Ile Tyr Ile Ser Asp Cys							
		525			530		535	540
	gtg ggc cag cac gat ttc gat ctc ttg aag cga ctc gtc ttg cct gac							1801
	Val Gly Gln His Asp Phe Asp Leu Leu Lys Arg Leu Val Leu Pro Asp							
		545			550		555	
35	ggc tcg att ttg agg tgt gag cac tat gca ctc cca act cgt gac cgt							1849
	Gly Ser Ile Leu Arg Cys Glu His Tyr Ala Leu Pro Thr Arg Asp Arg							
		560			565		570	
	ctc ttt gaa gac cct ctt cat gat ggc aaa acc atg ctc aag att tgg							1897
	Leu Phe Glu Asp Pro Leu His Asp Gly Lys Thr Met Leu Lys Ile Trp							

	575	580	585	
	aac ttg aac aag tac act gga att att gga gca ttc aac tgc caa gga			1945
	Asn Leu Asn Lys Tyr Thr Gly Ile Ile Gly Ala Phe Asn Cys Gln Gly			
	590	595	600	
5	gga gga tgg tgc aga gaa acc cga cgc aac caa tgc ttc tcc caa tgc			1993
	Gly Gly Trp Cys Arg Glu Thr Arg Arg Asn Gln Cys Phe Ser Gln Cys			
	605	610	615	620
	gtt aac acg tta acc gcc aca aca aat cct aag gac gtt gaa tgg aac			2041
	Val Asn Thr Leu Thr Ala Thr Thr Asn Pro Lys Asp Val Glu Trp Asn			
10	625	630	635	
	agt ggg aac aac cca atc tcc gtt gaa aac gtt gaa gag ttt gct ttg			2089
	Ser Gly Asn Asn Pro Ile Ser Val Glu Asn Val Glu Glu Phe Ala Leu			
	640	645	650	
15	ttc ttg tct cag tct aag aag ctt gtg ttg tct gga cca aac gat gat			2137
	Phe Leu Ser Gln Ser Lys Lys Leu Val Leu Ser Gly Pro Asn Asp Asp			
	655	660	665	
	ctc gag atc act ttg gag cct ttc aag ttt gag cta atc act gtc tca			2185
	Leu Glu Ile Thr Leu Glu Pro Phe Lys Phe Glu Leu Ile Thr Val Ser			
	670	675	680	
20	cca gtt gtc act att gag ggt agt tcg gtt cag ttt gct cca atc gga			2233
	Pro Val Val Thr Ile Glu Gly Ser Ser Val Gln Phe Ala Pro Ile Gly			
	685	690	695	700
	ttg gtt aac atg cta aac act agc ggt gca att cga tcc ttg gtg tat			2281
	Leu Val Asn Met Leu Asn Thr Ser Gly Ala Ile Arg Ser Leu Val Tyr			
25	705	710	715	
	cat gag gaa tcc gtt gag att gga gtt cgt ggt gct gga gag ttc agg			2329
	His Glu Glu Ser Val Glu Ile Gly Val Arg Gly Ala Gly Glu Phe Arg			
	720	725	730	
	gtt tat gca tca agg aaa cct gcg agc tgc aaa att gat ggt gaa gtt			2377
30	Val Tyr Ala Ser Arg Lys Pro Ala Ser Cys Lys Ile Asp Gly Glu Val			
	735	740	745	
	gtt gag ttt gga tac gaa gag tca atg gtg atg gtt caa gtg cct tgg			2425
	Val Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp			
	750	755	760	
35	tct gca ccc gag ggt ttg tct tct att aag tat gag ttt tag agtttccga			2476
	Ser Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Glu Phe			
	765	770	775	
	aggtgcttat ttgtatcctt ctaaactcct taattatgag ctccgtgccg tttctttttc			2536
	tatatgggttt ctgagagtga acatctaata ttaccact agggtataat tattggcttt			2596

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<210> 7

5 <211> 572

<212> PRT

<213> Brassica napus

<400> 7

10 Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp Cys  
5 10 15  
Thr Trp Asp Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His Lys  
20 25 30  
Gly Val Lys Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val Leu  
15 35 40 45  
Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile Asp  
50 55 60  
Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys Arg  
65 70 75 80  
20 Leu Pro Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser Pro  
85 90 95  
Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu Lys  
100 105 110  
Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu Cys  
115 120 125  
25 Gly Tyr Trp Gly Gly Leu Arg Pro Gly Ala Pro Thr Leu Pro Pro Ser  
130 135 140  
Thr Ile Val Arg Pro Glu Leu Ser Pro Gly Leu Lys Leu Thr Met Gln  
145 150 155 160  
30 Asp Leu Ala Val Asp Lys Ile Ile Asp Thr Gly Ile Gly Phe Val Ser  
165 170 175  
Pro Asp Met Ala Asn Glu Phe Tyr Glu Gly Leu His Ser His Leu Gln  
180 185 190  
Asn Val Gly Ile Asn Gly Val Lys Val Asp Val Ile His Ile Leu Glu  
195 200 205  
35 Met Leu Cys Glu Lys Tyr Gly Gly Arg Val Asp Leu Ala Lys Ala Tyr  
210 215 220  
Phe Lys Ala Leu Thr Ser Ser Val Asn Lys His Phe Asp Gly Asn Ala  
225 230 235 240

	Val	Ile	Ala	Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	
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	Glu	Ala	Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	
				260					265					270			
5	Pro	Ser	Gly	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	Met	
			275					280					285				
	Val	His	Cys	Ala	Tyr	Asn	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	Gln	Pro	
		290					295					300					
	Asp	Trp	Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Glu	Phe	His	Ala	
10	305					310					315				320		
	Ala	Ser	Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Ile	Ser	Asp	Cys	Val	
				325						330					335		
	Gly	Gln	His	Asp	Phe	Asp	Leu	Leu	Arg	Arg	Leu	Val	Leu	Pro	Asp	Gly	
			340						345					350			
15	Ser	Ile	Leu	Arg	Cys	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Arg	Leu	
		355						360					365				
	Phe	Glu	Asp	Pro	Leu	His	Asp	Gly	Lys	Thr	Met	Leu	Lys	Ile	Trp	Asn	
		370					375				380						
	Leu	Asn	Lys	Tyr	Thr	Gly	Ile	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	
20	385					390					395				400		
	Gly	Trp	Cys	Arg	Glu	Thr	Arg	Arg	Asp	Gln	Cys	Phe	Ser	Gln	Cys	Val	
				405						410					415		
	Asn	Thr	Leu	Thr	Ala	Thr	Thr	Asn	Pro	Asn	Asp	Val	Glu	Trp	Asn	Ser	
			420						425					430			
25	Gly	Asn	Asn	Pro	Ile	Ser	Ile	Glu	Asn	Val	Glu	Glu	Phe	Ala	Leu	Phe	
		435						440					445				
	Leu	Ser	Gln	Ser	Lys	Lys	Leu	Val	Leu	Ser	Gly	Gln	Asn	Asp	Asp	Leu	
		450					455					460					
	Glu	Ile	Thr	Leu	Glu	Pro	Phe	Lys	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	
30	465					470					475				480		
	Val	Val	Thr	Ile	Glu	Gly	Ser	Ser	Val	Gln	Phe	Ala	Pro	Ile	Gly	Leu	
				485						490					495		
	Val	Asn	Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Arg	Ser	Leu	Val	Tyr	His	
			500						505					510			
35	Glu	Glu	Ser	Val	Glu	Ile	Gly	Val	Arg	Gly	Ala	Gly	Glu	Phe	Arg	Val	
		515						520					525				
	Tyr	Ala	Ser	Lys	Lys	Pro	Val	Ser	Cys	Lys	Ile	Asp	Gly	Glu	Asp	Val	
		530					535					540					
	Glu	Phe	Gly	Tyr	Glu	Glu	Ser	Met	Val	Met	Val	Gln	Val	Pro	Trp	Ser	

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 Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Leu Phe  
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5    <210> 8  
      <211> 1762  
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      <213> Brassica napus

10   <220>  
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 Leu Glu Glu Lys Thr Pro Pro Gly Ile Val Asp Lys Phe Gly Trp Cys  
                          5                      10                      15  
 acg tgg gat gcg ttt tat ttg acg gtg aac cct gac gga gtt cat aag    96  
 Thr Trp Asp Ala Phe Tyr Leu Thr Val Asn Pro Asp Gly Val His Lys  
 20                      20                      25                      30  
 ggt gtt aag tgt ctc gtc gac ggt ggt tgt ccg ccg gga ttg gtc cta    144  
 Gly Val Lys Cys Leu Val Asp Gly Gly Cys Pro Pro Gly Leu Val Leu  
                          35                      40                      45  
 atc gac gac ggt tgg caa tcg att gga cat gac tcc gat ggt atc gat    192  
 25   Ile Asp Asp Gly Trp Gln Ser Ile Gly His Asp Ser Asp Gly Ile Asp  
                          50                      55                      60  
 gtt gaa ggg atg agt tgt acc gtc gcc ggg gag caa atg cct tgc agg    240  
 Val Glu Gly Met Ser Cys Thr Val Ala Gly Glu Gln Met Pro Cys Arg  
                          65                      70                      75                      80  
 30   ctt ccg aaa ttt caa gag aac ttc aag ttc aga gac tac gtc tct ccg    288  
 Leu Pro Lys Phe Gln Glu Asn Phe Lys Phe Arg Asp Tyr Val Ser Pro  
                          85                      90                      95  
 aaa gac aaa aac gaa gtc ggg atg aaa gct ttc gtc aga gat ctg aaa    336  
 Lys Asp Lys Asn Glu Val Gly Met Lys Ala Phe Val Arg Asp Leu Lys  
 35                      100                      105                      110  
 gaa gaa ttc tcc acc gtt gat tac atc tac gtc tgg cac gcg ctt tgc    384  
 Glu Glu Phe Ser Thr Val Asp Tyr Ile Tyr Val Trp His Ala Leu Cys  
                          115                      120                      125  
 ggy tac tgg ggw ggt ctt cgt ccc gga gct cct act ctt ccg ccs tcr    432

	Gly	Tyr	Trp	Gly	Gly	Leu	Arg	Pro	Gly	Ala	Pro	Thr	Leu	Pro	Pro	Ser	
	130						135					140					
	act	att	gtc	cgr	cca	gag	ctc	tcg	ccg	ggg	ctt	aag	ttg	acg	atg	caa	480
5	Thr	Ile	Val	Arg	Pro	Glu	Leu	Ser	Pro	Gly	Leu	Lys	Leu	Thr	Met	Gln	
	145					150					155					160	
	gat	ctc	gcc	gtt	gat	aag	atc	atc	gat	acc	gga	atc	gga	ttc	gtc	tcg	528
	Asp	Leu	Ala	Val	Asp	Lys	Ile	Ile	Asp	Thr	Gly	Ile	Gly	Phe	Val	Ser	
					165					170					175		
10	ccg	gac	atg	gcg	aac	gag	ttt	tac	gaa	ggt	ctt	cac	tct	cat	ctt	caa	576
	Pro	Asp	Met	Ala	Asn	Glu	Phe	Tyr	Glu	Gly	Leu	His	Ser	His	Leu	Gln	
				180					185					190			
	aac	gtc	ggc	att	aac	ggc	gtt	aaa	gtt	gac	gtt	atc	cac	ata	ctg	gag	624
	Asn	Val	Gly	Ile	Asn	Gly	Val	Lys	Val	Asp	Val	Ile	His	Ile	Leu	Glu	
			195					200					205				
15	atg	ttg	tgc	gag	aaa	tat	ggc	ggg	aga	gtt	gac	ttg	gct	aaa	gct	tac	672
	Met	Leu	Cys	Glu	Lys	Tyr	Gly	Gly	Arg	Val	Asp	Leu	Ala	Lys	Ala	Tyr	
	210					215					220						
	ttc	aag	gcg	tta	acg	tcg	tca	gtg	aat	aag	cat	ttt	gac	ggc	aac	gcc	720
	Phe	Lys	Ala	Leu	Thr	Ser	Ser	Val	Asn	Lys	His	Phe	Asp	Gly	Asn	Ala	
20	225					230					235					240	
	gtt	atc	gcc	agc	atg	gag	cac	tgt	aat	gac	ttc	atg	ttc	ctt	gga	acc	768
	Val	Ile	Ala	Ser	Met	Glu	His	Cys	Asn	Asp	Phe	Met	Phe	Leu	Gly	Thr	
					245					250				255			
25	gaa	gcc	atc	tct	cta	ggt	cgt	gtc	ggt	gat	gac	ttt	tgg	tgc	acg	gat	816
	Glu	Ala	Ile	Ser	Leu	Gly	Arg	Val	Gly	Asp	Asp	Phe	Trp	Cys	Thr	Asp	
				260					265					270			
	cca	tct	ggc	gac	att	aac	ggc	acg	tat	tgg	ctg	caa	gga	tgt	cac	atg	864
	Pro	Ser	Gly	Asp	Ile	Asn	Gly	Thr	Tyr	Trp	Leu	Gln	Gly	Cys	His	Met	
			275				280						285				
30	gtc	cac	tgt	gcc	tac	aac	agt	ctt	tgg	atg	gga	aat	ttc	atc	cag	cct	912
	Val	His	Cys	Ala	Tyr	Asn	Ser	Leu	Trp	Met	Gly	Asn	Phe	Ile	Gln	Pro	
			290				295					300					
	gat	tgg	gac	atg	ttt	cag	tcc	aca	cat	cct	tgt	gct	gag	ttc	cat	gct	960
	Asp	Trp	Asp	Met	Phe	Gln	Ser	Thr	His	Pro	Cys	Ala	Glu	Phe	His	Ala	
35	305					310					315					320	
	gct	tca	cgt	gcc	atc	tcc	ggg	ggg	ccc	att	tac	atc	agc	gat	tgt	gtg	1008
	Ala	Ser	Arg	Ala	Ile	Ser	Gly	Gly	Pro	Ile	Tyr	Ile	Ser	Asp	Cys	Val	
					325					330				335			
	ggc	cag	cac	gat	ttc	gat	ctc	ttg	agg	aga	ctc	gtt	ttg	cct	gac	ggg	1056

	Gly	Gln	His	Asp	Phe	Asp	Leu	Leu	Arg	Arg	Leu	Val	Leu	Pro	Asp	Gly	
				340					345					350			
	tcg	att	ttg	agg	tgt	gag	tac	tat	gct	ctc	cca	act	cgt	gac	cgt	ctc	1104
5	Ser	Ile	Leu	Arg	Cys	Glu	Tyr	Tyr	Ala	Leu	Pro	Thr	Arg	Asp	Arg	Leu	
			355					360					365				
	ttt	gaa	gac	cct	ctt	cat	gat	ggc	aaa	acc	atg	ctc	aag	att	tgg	aac	1152
	Phe	Glu	Asp	Pro	Leu	His	Asp	Gly	Lys	Thr	Met	Leu	Lys	Ile	Trp	Asn	
			370				375					380					
10	ttg	aac	aag	tac	act	gga	atc	atc	gga	gca	ttc	aac	tgt	caa	gga	gga	1200
	Leu	Asn	Lys	Tyr	Thr	Gly	Ile	Ile	Gly	Ala	Phe	Asn	Cys	Gln	Gly	Gly	
						385	390				395				400		
	gga	tgg	tgc	aga	gaa	act	cga	cgc	gac	caa	tgc	ttc	tcc	caa	tgc	gtt	1248
	Gly	Trp	Cys	Arg	Glu	Thr	Arg	Arg	Asp	Gln	Cys	Phe	Ser	Gln	Cys	Val	
				405					410				415				
15	aac	acg	tta	acc	gcc	aca	aca	aat	cct	aat	gac	gtt	gaa	tgg	aac	agt	1296
	Asn	Thr	Leu	Thr	Ala	Thr	Thr	Asn	Pro	Asn	Asp	Val	Glu	Trp	Asn	Ser	
				420				425					430				
	ggg	aac	aac	cgc	atc	tcc	att	gaa	aac	gtt	gaa	gag	ttt	gct	ttg	ttc	1344
	Gly	Asn	Asn	Pro	Ile	Ser	Ile	Glu	Asn	Val	Glu	Glu	Phe	Ala	Leu	Phe	
20			435				440				445						
	ttg	tct	caa	tcc	aag	aag	ctt	gtg	ttg	tcc	ggg	caa	aac	gat	gat	ctc	1392
	Leu	Ser	Gln	Ser	Lys	Lys	Leu	Val	Leu	Ser	Gly	Gln	Asn	Asp	Asp	Leu	
			450				455				460						
25	gag	atc	aca	tta	gag	ccc	ttc	aag	ttc	gag	ctc	atc	act	gtc	tca	cca	1440
	Glu	Ile	Thr	Leu	Glu	Pro	Phe	Lys	Phe	Glu	Leu	Ile	Thr	Val	Ser	Pro	
			465			470			475				480				
	gtt	gtc	acc	att	gag	ggc	agt	tcg	gtt	cag	ttt	gct	cca	atc	gga	ttg	1488
	Val	Val	Thr	Ile	Glu	Gly	Ser	Ser	Val	Gln	Phe	Ala	Pro	Ile	Gly	Leu	
				485			490				495						
30	gtt	aac	atg	ctt	aac	act	agc	ggt	gcg	att	cga	tcc	ttg	gtt	tat	cat	1536
	Val	Asn	Met	Leu	Asn	Thr	Ser	Gly	Ala	Ile	Arg	Ser	Leu	Val	Tyr	His	
				500				505					510				
	gag	gaa	tcc	gtt	gag	atc	ggt	gtt	cgt	ggt	gct	gga	gaa	ttc	agg	gtt	1584
35	Glu	Glu	Ser	Val	Glu	Ile	Gly	Val	Arg	Gly	Ala	Gly	Glu	Phe	Arg	Val	
			515				520				525						
	tat	gca	tcg	aag	aaa	cct	gtg	agc	tgc	aag	att	gat	ggt	gaa	gat	gtt	1632
	Tyr	Ala	Ser	Lys	Lys	Pro	Val	Ser	Cys	Lys	Ile	Asp	Gly	Glu	Asp	Val	
			530			535					540						
	gag	ttt	ggg	tac	gaa	gag	tca	atg	gtg	atg	gtt	caa	gtg	cct	tgg	tct	1680



Glu Phe Gly Tyr Glu Glu Ser Met Val Met Val Gln Val Pro Trp Ser  
 545 550 555 560  
 gca cca gag ggt ttg tct tct att aag tat ttg ttt tag agttatttaa 1729  
 Ala Pro Glu Gly Leu Ser Ser Ile Lys Tyr Leu Phe

5 565 570  
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<211> 25

10 <212> DNA

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ccaatctgat catgcttggt ccgaa 25

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30 <210> 11

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<400> 11

ctaccaaatt ccacaactta aagttca 27

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20 <400> 13  
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<400> 14  
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35 <210> 15  
<211> 30  
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<213> Artificial Sequence

&lt;220&gt;

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 15

5      ttggaagaga agacgccgcc gggaatcgtc

30

&lt;210&gt; 16

<211> 30

## ⟨212⟩ DNA

## 10 <213> Artificial Sequence

$\langle 220 \rangle$

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

15       $\langle 400 \rangle$     16

ttaagccccg gcgagagctc tggccggaca

30

&lt;210&gt; 17

&lt;211&gt; 30

20      &lt;212&gt; DNA

### <213> Artificial Sequence

**<220>**

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

25

&lt;400&gt; 17

accaatccaa aatctcatca aataatcgca

30

&lt;210&gt; 18

30       $\langle 211 \rangle$  25

## <212> DNA

### <213> Artificial Sequence

 $\langle 220 \rangle$ 

35 <223> Designed oligonucleotide primer to obatin raffinose synthase gene.

<400> 18

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30

<210> 19  
<211> 29  
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5

<220>  
<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 19  
10 atggctccac cgagcgtaat taaatccga 29

<210> 20  
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15 <213> Artificial Sequence

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20 <400> 20  
ctaaaactca tacttaatag aagacaaacc 30

<210> 21  
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25 <212> DNA  
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30 n is i.

<400> 21  
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35 <210> 22  
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n is i, r is a or g.

5 <400> 22

ggccacatnt tnacnarncc natngngnch aa 32

<210> 23

<211> 30

10 <212> DNA

<213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

15

<400> 23

tgttactagg cgaaacaaga gtagctctga 30

<210> 24

20 <211> 47

<212> DNA

<213> Artificial Sequence

<220>

25 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,  
n is i.

<400> 24

30 cgaggnggnt gncncncngg nttngtnatn atngangang gntggca 47

<210> 25

<211> 29

<212> DNA

<213> Artificial Sequence

35

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene,  
n is i, y is t or c, r is a or g.

- <400> 25  
atyttrtcna cngcnarrtc ytccatngt 29
- <210> 26  
5 <211> 38  
<212> DNA  
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- <220>  
10 <223> Designed oligonucleotide primer to obtain raffinose synthase gene,  
n is i, y is t or c.
- <400> 26  
15 ggnacntant ggytncangg ntgncanatg gtncantg 38
- <210> 27  
<211> 32  
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- 20 <220>  
<223> Designed oligonucleotide primer to obtain raffinose synthase gene, April, 16,  
n is <sup>i</sup> r is a or g. E.W.
- 25 <400> 27  
ggccacatnt tnacnarncc natnggngcn aa 32 April, 16,  
K.O.
- <210> 28  
30 <211> 26  
<212> DNA  
<213> Artificial Sequence
- <220>  
35 <223> Designed oligonucleotide primer to obtain raffinose synthase gene.
- <400> 28  
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- <210> 29

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<212> DNA  
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<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 32

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5

<210> 33

<211> 28

<212> DNA

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10

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<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 33

15 gacatgttcc catctacaca cccttgtg 28

<210> 34

<211> 30

<212> DNA

20 <213> Artificial Sequence

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25 <400> 34

ccaatttatg ttagtgatgt tgttggcaag 30

<210> 35

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30 <212> DNA

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tgcactccca gggtagaatt gtcatac 26

<210> 36



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5 <220>  
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 n is i.

10 <400> 36  
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<210> 37  
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 15 <213> Artificial Sequence

<220>  
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 n is i, r is a or g.

20 <400> 37  
 cantgnacca tntgncancc ntgnarccan tangtncc 38

<210> 38  
 25 <211> 30  
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35 <210> 39  
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Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

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<210> 40

<211> 30

<212> DNA

10 <213> Artificial Sequence

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

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15 ggattcgaca caaaccgcca cgtcatcgtc 30

<210> 41

<211> 27

20 <212> DNA

<213> Artificial Sequence

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<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

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<400> 41

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<210> 42

30 <211> 30

<212> DNA

<213> Artificial Sequence

<220>

35 <223> Designed oligonucleotide primer to obtain raffinose synthase gene.

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<210> 43  
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<210> 44  
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20 tctacgtctg gcacgcgctt tgcggctac 29  
  
<210> 45  
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gttgacgtca tccacatatt ggagatgttg t 31  
  
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<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 46

gttatcgcta gcatggagca ctgtaatga

29

5

<210> 47

<211> 35

<212> DNA

<213> Artificial Sequence

10

<220>

<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

<400> 47

15

aacgagctca atccaaaatc tcatcaaata atcgc

35

<210> 48

<211> 25

<212> DNA

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<213> Artificial Sequence

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<223> Designed oligonucleotide primer to obtain raffinose synthase gene.

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25

<210> 49

<211> 25

30

<212> DNA

<213> Artificial Sequence

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<223> Designed oligonucleotide linker to obtain raffinose synthase gene.

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<400> 49

gatcgagctc gtgtcggatc cagct

25

<210> 50

<211> 17  
<212> DNA  
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5 <220>  
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<400> 50  
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10  
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raffinose synthase gene.

20 <400> 51  
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<210> 52  
<211> 30  
25 <212> DNA  
<213> Artificial Sequence

<220>  
<223> Designed oligonucleotide primer to confirm direction of the inserted  
30 raffinose synthase gene.

<400> 52  
ggattcgaca caaaccgcca cgtcatcgtc 30

35 <210> 53  
<211> 29  
<212> DNA  
<213> Artificial Sequence

$\langle 220 \rangle$

<223> Designed oligonucleotide primer to confirm direction of the inserted raffinose synthase gene.

5

<400> 53

tctacgtctg gcacgcgctt tgcggctac

29

[illegible]